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Unexpected distribution of *qnrB* gene in *E. coli* isolates from different origins in Ecuador

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Summary

Fluoroquinolone resistance can be conferred through chromosomal mutations or acquisition of plasmids that carry genes such as *qnr*. In this study 3,201 strains of commensal *Escherichia coli* were isolated from: 1) 2,374 (74%) strains from humans and chickens from rural northern coastal Ecuador, 2) 827 (26%) strains from chickens from an Ecuadorian industrial poultry operation. We also analyzed 114 fluoroquinolone resistant strains from patients with urinary infection attending three urban hospitals in Quito Ecuador. Isolates were subjected to antibiotic susceptibility screening, and fluoroquinolone resistant isolates (FRIs) were screened for the presence of *qnrB* genes. We found significantly higher phenotypic resistance to fluoroquinolones in *E. coli* from chickens in both the rural area (22%) and from the industrial operation (10%) than those from humans in the rural communities (3%). However *E. coli* isolates from healthy humans in the rural communities had higher rates of *qnrB* genes (31%, 11 of 35 isolates) compared to chickens from industrial operations (6%, 3 of 81 isolates) and chickens from rural communities (2.8%, 7 of 251 isolates). In addition, human FRIs from urban hospitals had low occurrence of *qnrB* genes (0.87%, 1 of 114 isolates). These results suggest that the *qnrB* gene is more widely distributed in rural settings, where antibiotic usage is low, compared to urban hospital and industrial poultry settings. The role of *qnrB* genes in clinical resistance to fluoroquinolones is still unclear and requires more research.

Keywords

Escherichia coli; *qnrB*; quinolone resistance

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Competing interests

The authors declare that they have no competing interest

Introduction

Antibiotic resistance is an increasingly serious public health issue. Every year in the United States alone, more than two million people are infected with antibiotic resistant bacteria, resulting in more than 23,000 deaths [3]. Both medical and agricultural usage of antibiotics has been linked to antibiotic resistance in pathogens [6, 7, 15, 16]. Resistance to fluoroquinolones is particularly troubling because they are broad-spectrum antibiotics used to treat serious bacterial infections, especially those acquired in hospitals [2, 3, 5]. Resistance to fluoroquinolones can be caused by mutations in chromosomal genes [2] and by the presence of conjugative or non-conjugative plasmids carrying *qnr* or other genes [10, 21, 27]. Plasmid-mediated quinolone resistance in human pathogens has been associated with food-producing animals [8, 28], although this claim has been challenged by other studies [20]. Additionally, *qnrB* genes have been found widely distributed in South American countries [17, 18, 20]. In Ecuador, where there is no restriction on the use of fluoroquinolones in food animals, the prevalence of fluoroquinolone resistance in community-acquired urinary tract *E. coli* isolates is reported to be 41% [22]. In this study, we assess fluoroquinolone resistance and the presence of *qnrB* genes in *E. coli* isolates obtained from fecal samples of chickens and humans in a rural, low antibiotic use setting to fecal samples obtained in two higher antibiotic use settings. Specifically, we compare fluoroquinolone resistant isolates (FRIs) from 1) healthy human individuals from rural communities, 2) chickens (broiler and free range) from rural communities, 3) humans from urban hospitals, and 4) chickens from an industrial poultry operation in Ecuador.

Materials and methods

Samples and bacterial isolates

E. coli was isolated from 1,167 human fecal samples and 1,207 chicken cloacal swabs (from 1,134 chickens) cultured on MacConkey agar. Five lactose-fermenting colonies were selected from each sample and tested for glucuronidase activity on Chromocult agar; glucuronidase positive colonies were subjected to antibiotic susceptibility tests and 1 FRI was selected from each sample. FRIs showed an inhibition zone to 20 mm using discs with 5 µg ciprofloxacin and Kirby-Bauer antibiotic susceptibility testing, in accordance with the Clinical and Laboratory Standards Institute guidelines.

Isolates from chickens in rural communities

We obtained 1,207 *E. coli* isolates from chickens in a rural community involved with small-scale poultry farming operations in northwestern Ecuador from January to March 2009. The majority of these isolates (961; 80%) were from broiler chickens raised in small-scale commercial poultry operations. These birds were purchased from a local distributor and fed with commercial poultry feed. Of these 961 isolates from broiler chickens, 831 came from 30 chickens on three farms in one community sampled weekly for six weeks, and 124 came from 25 chickens sampled once cross-sectionally from another farm in the same community. In addition, another 246 (20%) isolates were from household varieties (other breeds of chickens also purchased from a local distributor). Isolates from all chickens in rural

communities were labeled RemCHK. In addition, one isolate (Rem6) was isolated from water extracted from a well in the same community where we collected chicken isolates.

Isolates from humans in rural communities

We obtained 1,167 commensal *E. coli* isolates from healthy humans (controls) participating in a case-control study of diarrheal diseases from 24 communities in northwestern Ecuador between February 2009 to February 2010. Details about the region, study design and sampling strategy were described previously [4]. Human isolates were labeled RemHUM. All interaction with human subjects was approved by the University of Michigan's Institutional Review Board and Universidad San Francisco de Quito's bioethics committee.

Isolates from chickens in an industrial operation

We obtained 827 *E. coli* isolates from an industrial poultry operation (located in the Ecuadorian Coast approximately 300 kilometers from the study region), from broiler chickens sampled between March to November 2010. Isolates from these industrial chickens were labeled IndCHK. These animals were kept in coops and received oxytetracycline in the drinking water (10mg/litre).

Isolates from humans in an urban hospitals

We obtained 114 clinical FRI *E. coli* from patients with urinary infections collected in three hospitals in urban Quito from May to July 2010. Of these isolates, 61 were from Hospital Vozandes (kindly provided by Jeannette Zurita), 42 were from Hospital Carlos Andrade Marín (kindly provided by Isabel Narváez), and 11 were from the Institute of Microbiology at Universidad San Francisco de Quito. These clinical human isolates were labeled *E. coli* Quito.

Polymerase chain reaction amplification and DNA sequence analysis

Bacterial DNA from a single FRI colony was released from cells using a boiling technique [25]. A PCR reaction for *qnrB* genes was carried out following [8], using primers to amplify internal fragments of the target genes: *qnrB* F 5'-GGMATHGAAATTCGCCACTG-3' and *qnrB* R 5'-TTTGCYGYCYGCCAGTCGAA-3'. PCR conditions were as follows: 95 °C for 5 min, 35 cycles of 94 °C for 30 sec, 56 °C for 40 sec and 72 °C for 1 min, and a final incubation at 72 °C for 10 min. Amplicons were sequenced by the University of Michigan DNA Sequencing Core. The *qnrB* gene sequences described in this report comprise accession numbers JN714812 to JN714838 (Table 2). A subset of these amplicons (IndCHK24, IndCHK25, IndCHK26 and *E. coli* Quito Hospital) were sequenced a second time at Functional Biosciences, Inc, Madison, WI to rule out mistakes and they were used for phylogenetic analysis using Mega5.1 program (Figure 1). Two *qnrB* sequences from broiler chicken FRIs came from the same animal, from two colonies collected two weeks apart (RemCHKb7 y RemCHKb9).

Statistical analysis

A χ^2 test was used to analyze the differences in fluoroquinolone resistance and *qnrB* gene frequency between the sample types.

Results and Discussion

Rates of resistance

Significantly higher rates of resistance to fluoroquinolones ($p < 0.001$) were observed in isolates from chickens in rural communities (broilers 214 of 961 isolates, 22.3% and household varieties: 37 of 246 isolates, 15%) when compared with isolates from chickens in industrial operations (81 of 827 isolates, 9.8%) or with human isolates from rural communities (35 of 1,167 isolates, 3%).

Presence of *qnrB* gene in fluoroquinolone-resistant isolates

There was a substantial difference in the percentage of human FRIs carrying *qnrB* genes depending on the origin of the isolates: 31% (11 of 35 isolates) of human FRIs from rural communities carried the *qnrB* gene versus 0.87% (1 of 114 isolates) of FRIs from hospitals in Quito ($p < 0.0001$) (Table 1). Differences were less evident in chicken isolates: 1.87% (4 of 214 isolates) of FRIs from broiler chickens in rural communities and 8% (3 of 37 isolates) from household varieties carried the *qnrB* gene, versus 6% (3 of 81 isolates) in FRIs from broiler chickens from the industrial operation ($p = 0.4$). No significant differences were observed between FRIs from broiler chickens and household varieties in the rural community ($p = 0.069$).

Phylogenetic analysis of *qnrB* genes

Nucleotide sequences of all the amplicons showed high similarity to previously described *qnrB* genes Figure 1.

Conclusions

Based on the published literature [1] and the high prevalence of fluoroquinolone resistance reported in Ecuador [22], we expected to encounter a greater presence of *qnrB* genes in pathogenic FRIs from hospitals in Quito where antibiotic use is high, compared to rural settings where antibiotic use is low. On the contrary, where we found a low overall prevalence of FRI, in commensal *E. coli* isolated from humans in rural communities 3%, a high proportion of these carried *qnrB* genes 31%. Where we found a high prevalence of FRIs, chickens from these rural communities, a low proportion of these carried *qnrB* genes 2.7%. The lowest proportion of *qnrB* gene carriage was found in FRI from urban hospitals in Quito 0.9%, where antibiotic use is high. Although *qnr* genes cause low-level resistance to quinolones [9], they are thought to be important in the development of the highly resistant phenotype [14]. Therefore it was surprising to find a lack of association of the *qnrB* gene with clinical fluoroquinolone resistance in this study.

Other studies have found that *qnrB* genes are widely distributed in commensal *E. coli* isolated from healthy humans including children in urban settings in Peru and Bolivia [17, 18], humans in remote Peruvian Amazon communities [19], and from domestic and farm animals in Germany [23]. We found a high rate of *qnrB* carriage in healthy humans from rural, but not urban areas of Ecuador. Despite high rates of fluoroquinolone resistance in chickens, we did not find high rates of *qnrB* carriage in the FRIs from these animals.

Although our findings may suggest that *qnrB* genes are not linked to fluoroquinolone clinical resistance, they have been associated with the development high resistance to quinolones [14, 24] and *qnrB* genes are one of the most common plasmid-mediated quinolone resistance genes [13]. It is also a concern the presence of *qnrB* genes in isolates from domestic animals as these genes have been found in food pathogens, such as *Salmonella* [11]. Additional research about the role of *qnr* genes in fluoroquinolone resistance is necessary. The main limitation of this study was the segment of DNA amplified which did not allow us to determine the diversity of *qnr* genes detected.

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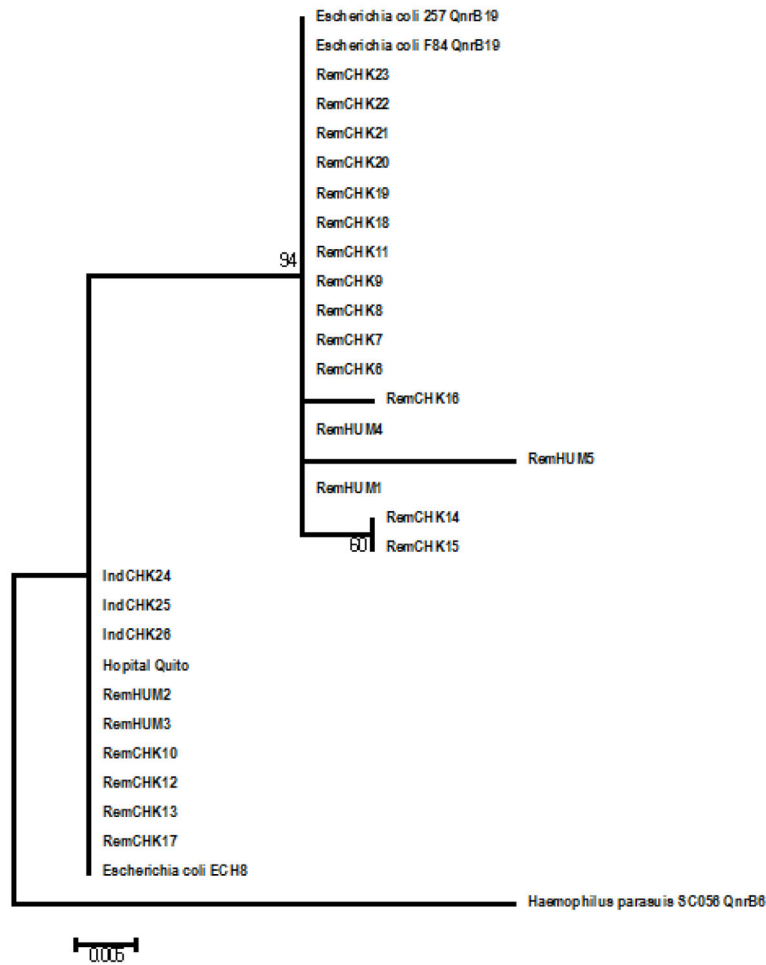


Fig. 1. Maximum likelihood analysis of *qnrB* genes: Bacterial sequences obtained from humans in Ecuadorian rural communities are named RemHUM; sequences from chickens in rural communities are RemCHK; *qnrB* from hospital isolates in Quito: *E. coli* Quito Hospital hospital and sequences from isolates from a poultry industrial operation are IndCHK. The rest of the sequences were obtained from GenBank: *E. coli* strain F84, accession number KM094204.1; *E. coli* strain F257 accession number KM094205.1; *E. coli* strain ECH8, accession number KP268825.1; *H parasuis* strain SC056, accession number HQ117877.1. Numbers are bootstrap values obtained after 500 pseudoreplicates. Asterisk indicates sequences analysed in this paper.

Table 1

Fluoroquinolone resistant isolates FRIs of *E. coli* with and without *qnrB*. Five *E. coli* isolates were analyzed from each fecal sample from either humans or chickens inhabiting rural communities in the northern coast of Ecuador.

Source of isolates	Number of isolates	FRIs %	FRIs with <i>qnrB</i> %	<i>p</i> -value
Human isolates from rural area	1,167	35 3.0%	11 31%	
Human isolates from urban hospitals	ND**	114	1 0.87%	<i>p</i> <0.0001
Broiler chicken isolates from rural area	961	214 22.3%	4 1.87 %	
Household varietals from rural area	246	37 15%	3 8.1%	
Broiler chicken isolates from an industrial poultry operation	827	81 9.8%	3 6.0%***	

** ND= not determined;

*** Only 50 isolates were analyzed for *qnrB* genes. P value obtained from comparison of human isolates from the rural community and the hospital in Quito

Table 2

Nucleotide sequences of *qnrB* genes from fluoroquinolone resistant isolates (FRIs) from humans and chickens inhabiting rural communities in the northern coast of Ecuador, FRIs from chicken industrial operation and a FRI from an urban hospital in Quito.

Amplicon	Gen Bank Accession number
RemHUM1	JN714812
RemHUM2	JN714813
RemHUM3	JN714814
RemHUM4	JN714815
RemHUM5	JN714816
RemCHK6	JN714817
RemCHK7	JN714818
RemCHK8	JN714819
RemCHK9	JN714820
RemCHK10	JN714821
RemCHK11	JN714822
RemCHK12	JN714823
RemCHK13	JN714824
RemCHK14	JN714825
RemCHK15	JN714826
RemCHK17	JN714828
RemCHK18	JN714829
RemCHK19	JN714830
RemCHK20	JN714831
RemCHK21	JN714832
RemCHK22	JN714833
RemCHK23	JN714834
IndCHK24	JN714835
IndCHK25	JN714836
IndCHK26	JN714837
Quito Hospital	JN714838